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Abstract: Molecular genetic analysis of five cases of 3p-syndrome (del(3)(qter-p25:)) was performed to investigate the relationship between the molecular pathology and clinical phenotype. Fluorescence in situ hybridization studies and analysis of polymorphic DNA markers from chromosome 3p25-p26 demonstrated that all four informative cases had distal deletions. However, the extent of the deletion was variable: in two patients with the most extensive deletions the deletion breakpoint mapped between RAF1 and D3S1250, in one patient the deletion breakpoint was between D3S1250 and D3S601, and in two patients the deletion commenced telomeric to D3S601 (and telomeric to D3S1317 in one of these). All five patients displayed the classical features of 3p- syndrome (mental retardation, growth retardation, microcephaly, ptosis and micrognathia) demonstrating that loss of sequences centromeric to D3S1317 is not required for expression of the characteristic 3p- syndrome phenotype. The three patients with the most extensive deletions had cardiac septal defects suggesting that a gene involved in normal cardiac development is contained in the interval D3S1250 and D3S18. The PMCA2 gene is contained within this region and deletion of this gene may cause congenital heart defects. At least three patients were deleted for the von Hippel - Lindau (VHL) disease gene although none had yet developed evidence of VHL disease. We conclude that molecular analysis of 3p- syndrome patients enhances the management of affected patients by identifying those at risk for VHL disease, and can be used to elucidate the critical regions for the 3p- syndrome phenotype

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Molecular genetic analysis of the 3p– syndrome

Maude E.Phipps, Farida Latif[1], Amanda Prowse, Stewart J.Payne[2], Jeanne Dietz-Band[3], Margaret Leversha, Nabeel A.Affara, Anthony T.Moore[4], John Tolmie[5], Albert Schinzel[6], Michael I.Lerman[1], Malcolm A.Ferguson-Smith and Eamonn R.Maher*

Cambridge University Department of Pathology, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK, [1]Laboratory of Immunobiology, National Cancer Institute, Frederick Cancer Facility, Frederick, MD, USA, [2]Molecular Genetics Laboratory, Addenbrooke's Hospital, Cambridge, UK, [3]Oncor Inc, 209 Perry Parkway, Gaithersberg, MD 20877, USA, [4]Department of Ophthalmology, Addenbrooke's Hospital, Cambridge, [5]Duncan Guthrie Institute of Medical Genetics, Glasgow, UK and [6]Institute of Medical Genetics, University of Zurich, Switzerland

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Molecular genetic analysis of five cases of 3p– syndrome (del(3)(qter→p25:)) was performed to investigate the relationship between the molecular pathology and clinical phenotype. Fluorescence *in situ* hybridization studies and analysis of polymorphic DNA markers from chromosome 3p25–p26 demonstrated that all four informative cases had distal deletions. However, the extent of the deletion was variable: in two patients with the most extensive deletions the deletion breakpoint mapped between RAF1 and D3S1250, in one patient the deletion breakpoint was between D3S1250 and D3S601, and in two patients the deletion commenced telomeric to D3S601 (and telomeric to D3S1317 in one of these). All five patients displayed the classical features of 3p– syndrome (mental retardation, growth retardation, microcephaly, ptosis and micrognathia) demonstrating that loss of sequences centromeric to D3S1317 is not required for expression of the characteristic 3p– syndrome phenotype. The three patients with the most extensive deletions had cardiac septal defects suggesting that a gene involved in normal cardiac development is contained in the interval D3S1250 and D3S18. The PMCA2 gene is contained within this region and deletion of this gene may cause congenital heart defects. At least three patients were deleted for the von Hippel–Lindau (VHL) disease gene although none had yet developed evidence of VHL disease. We conclude that molecular analysis of 3p– syndrome patients enhances the management of affected patients by identifying those at risk for VHL disease, and can be used to elucidate the critical regions for the 3p– syndrome phenotype.

INTRODUCTION

Distal deletion of the short arm of chromosome 3 (3p– syndrome) has emerged as a chromosomal disorder with a recognizable phenotype. A total of 22 cases have been reported and apart from one mother and son pair, all cases have arisen *de novo* (1–5). Characteristic features include low birthweight, microcephaly, trigonocephaly, hypotonia, psychomotor and growth retardation, ptosis, telecanthus, downslanting palpebral fissures and micrognathia. Frequent but inconstant complications are postaxial polydactyly (nine of 22), renal anomalies (seven of 22), cleft palate, congenital heart defects (seven of 22), preauricular pits, sacral dimple and gastrointestinal anomalies. The von Hippel–Lindau (VHL) disease tumour suppressor gene maps to chromosome 3p25–p26 and patients hemizygous for the VHL disease gene would be expected to develop VHL-related tumours including retinal and cerebellar haemangioblastomas and renal cell carcinoma (6). We have investigated five patients with 3p– syndrome to define the molecular pathology of this disorder, and to determine the relationship between the phenotypic variation in 3p– syndrome and the extent and position of the 3p deletions.

RESULTS

Clinical studies

Case report. The previously unreported patient (CUMG3.4) was the first child of a 26-year-old mother and an unrelated 24-year-old father. Both parents were of Chinese origin. Pregnancy was uneventful and the patient was born at 37 weeks gestation with a birthweight of 2.3 kg. She was referred for investigation at 5 weeks of age because of poor feeding, inadequate weight gain and dysmorphic features. Chromosomal analysis showed 46XX(del(3)(qter→3p25:)) and normal parental karyotypes. Clinical findings in the proband included brachycephaly, ptosis, telecanthus, micrognathia, bilateral preauricular sinuses and severe hypotonia. Echocardiography at age 4 months demonstrated a large secundum atrial septal defect, but renal ultrasound and skeletal survey were normal. Follow up to age 6 years has shown severe developmental delay, microcephaly (3.5 SD below mean) and growth retardation (4 SD below mean).

Summary of clinical features. Table 1 contains details of the clinical features of the five 3p– syndrome patients we investigated.

Chromosome 3p deletion mapping

The results of the combined fluorescence *in situ* hybridization (FISH) and molecular genetic analysis are shown in Table 2, and the background genetic map in Figure 1. The four patients from whom lymphoblastoid cell lines were available were investigated by FISH analysis and each was deleted for the subtelomeric probes D3S1442, D3S1443 and D3S1444 indicating that all had subtelomeric deletions (see Figure 2). In one patient (CUMG3.10) a lymphoblastoid cell line was not available so FISH analysis

*To whom correspondence should be addressed

Table 1. Clinical features of five patients with 3p− syndrome investigated

Clinical features	Patients CUMG3.1	GM10922	CUMG3.4	GM10985	CUMG3.10
Sex	Male	Male	Female	Female	Female
Age (months)	120	13	72	8.5	88
Psychomotor retardation	+	+	+	+	+
Growth retardation	+	+	+	+	+
Microcephaly	+	+	+	+	+
Hypotonia	+	+	+	+	+
Ptosis	+	+	+	+	+
Hypertelorism/telecanthus	+	−	−	+	−
Preauricular pits	+	−	+	−	−
Micrognathia	+	+	+	+	+
Polydactyly	−	−	−	−	−
Congenital heart disease	Ventricular septal defect	Endocardial cushion defect	Atrial septal defect	−	−
Renal anomaly	−		−	+	−
Other	Hyperhidrosis	Hypothyroidism	−	Malrotation of the colon, moderate sensorineural deafness	Anal stenosis, absent ear lobes, trigonocephaly, pectus excavatum

Table 2. Results of deletion mapping in five patients with chromosome 3p− syndrome

Locus	Patient CUMG3.1	GM10922	CUMG3.4	GM10985	CUMG3.10
D3S651	+		+		+
RAF1	+	+	+		+
D3S732	−		+		
D3S1250	−	−	+	+	
D3S587	−	−	−	+	
D3S1038			−		+
D3S601	−	−	−	+	+
D3S1317					+
D3S18	−	−	−	−	
D3S1442	−	−	−	−	
D3S1443	−	−	−	−	
D3S1444	−	−	−	−	

+ = not deleted, − = deleted, no entry = not done or uninformative.

was not possible and the D3S1444 microsatellite polymorphism was uninformative. The centromeric extent of the deletions was variable. In CUMG3.1 and GM10922 the deletion commenced between RAF1 and D3S1250. CUMG3.4 was retained at D3S1250 but was deleted for more telomeric sequences. In two patients (GM10985 and CUMG3.10) the deletion commenced telomeric to D3S601 (and telomeric to D3S1317 in CUMG3.10). At least three patients (CUMG3.1, CUMG3.4 and GM10922) were deleted for the VHL gene (see Figure 1 and Table 2).

The three patients for which parental DNA was available were analysed using DNA polymorphisms at RAF1, D35732, D3S601, D3S18, D3S1250, D3S1038, D3S1317 and D3S1444 (see Table 3). CUMG 3.10 was heterozygous at three informative loci: D3S651, D3S1038 and D3S1317. Although molecular genetic analysis did not detect a chromosome 3p deletion in CUMG3.10, cytogenetic analysis had demonstrated an unequivocal deletion and the patient had the typical phenotypic features of 3p− syndrome (1). CUMG3.1 and CUMG3.4 were informative at D3S1038 and D3S18, and at D3S732 and D3S1250 respectively. In both cases the chromosome 3p deletion detected by FISH was confirmed and the deletion was shown to have occurred on the maternally inherited chromosome (see Figure 3).

A possible association was detected between the extent of the 3p deletion and the occurrence of congenital heart disease in 3p− syndrome. The three patients with deletions extending centromeric to D3S601 all had cardiac septal defects (ventricular septal defect in CUMG3.1, endocardial cushion defect in GM10922, and an atrial septal defect in CUMG3.4). There was no relationship between the extent of the deletion and the presence of preauricular pits.

DISCUSSION

We have reported the clinical features of a new patient with 3p− syndrome, and mapped the extent of the 3p deletion in five cases of 3p− syndrome. By using cosmid probes mapping to the subtelomeric region of chromosome 3p we demonstrated that all four informative patients had subtelomeric deletions; however, Mowrey *et al.* (5) reported another patient (not studied by us) with 3p− syndrome and an interstitial deletion of chromosome 3p25−p26. The centromeric extent of the chromosome 3p deletions were variable: in two patients the deletion commenced between RAF1 and D3S1250, in one between D3S1250 and D3S587, and in two patients the deletion breakpoint was telomeric

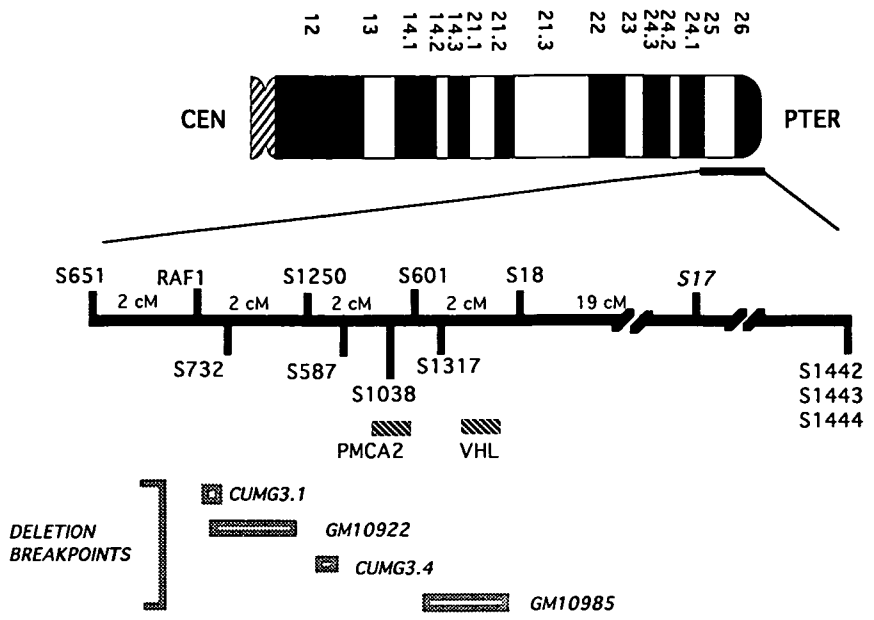

Figure 1. Map of chromosome 3p25−p26. The location of the four deletion breakpoints for which flanking markers were demonstrated are shown. The breakpoint in CUMG3.10 was telomeric to D3S1317, but this case was not informative at more telomeric loci.

Table 3. Details of FISH probes and polymorphic markers investigated

Locus	Fish probe	Polymorphism	Reference
D3S651	CI3-312	Microsatellite	(22)
RAF1	CRc129	*Taq*I	(11)
D3S732	LIB 4A-52	*Hin*dIII	(11)
D3S1250	cos 56	−	(12)
D3S587	cLIB 12-48	*Eco*RI	(16)
D3S1038	−	Microsatellite	(13)
D3S601	cos 7	*Taq*I	(11)
D3S1317	−	Microsatellite	(14)
D3S18	cLIB-1	*Bam*HI	(11)
D3S1444	3PT-B47-2G	Microsatellite	Dietz-Band unpublished observations
D3S1442	3PT-B47-5E	−	Dietz-Band unpublished observations
D3S1443	3PT-B47-2A	−	Dietz-Band unpublished observations

− = not investigated or not available

to D3S601 (and telomeric to D3S1317 in one of these). Mowrey *et al.* (5) had mapped the deletion breakpoints in GM10922 and GM10985 between RAF1 and D3S18, and we were able to sublocalize the deletion endpoints within this interval, and demonstrate that the deletions were different in the two patients. In addition to the five patients with 3p− syndrome, we have also mapped the chromosome 3p breakpoint in seven patients with inversions or translocations involving chromosome 3p25−p26 (8; unpublished observations). In two cases the translocation or inversion breakpoint mapped between RAF1 and D3S1250, between D3S1250 and D3S601 in one case; between D3S601 and D3S18 in two cases, and telomeric to D3S18 in two cases. The approximate genetic distance between RAF1 and D3S1250 is 2 cM, between D3S1250 and D3S601 is 2 cM, and between D3S601 and D3S18 is 2 cM (see Figure 1). Thus the distribution of cytogenetic breakpoints within the RAF1 to D3S18 interval is approximately proportional to the genetic distances between loci and does not provide evidence for a 'hot spot' for cytogenetic rearrangements within a specific subinterval.

The parental origin of *de novo* 3p deletions was defined in two cases, and was maternal in both cases. In a further three cases of *de novo* 3p− syndrome studied by Mowrey *et al.* (5), the deletion was of paternal origin in two cases and maternal in one. Genomic imprinting of genes within chromosome 3p25−p26 has not been reported and we were unable to discern any effect of the parent of origin of the 3p deletion on the clinical phenotype. In 4p− and 5p− syndromes (Wolf−Hirschhorn and cri-du-chat syndromes respectively), 80% of *de novo* deletions arise in the paternal germline (reviewed in reference 19). Further investigations are required to determine whether the differences in the parental origin of 3p−, 4p− and 5p− syndrome patients is a real finding or reflects the small number of 3p cases in which the parental origin has been defined.

All five patients investigated displayed typical features of 3p− syndrome, so it appears that the classical features of 3p− syndrome (psychomotor and growth retardation, dysmorphological features) result from loss of genes telomeric to D3S1317. Mowrey *et al.* (5) reported that a patient with an

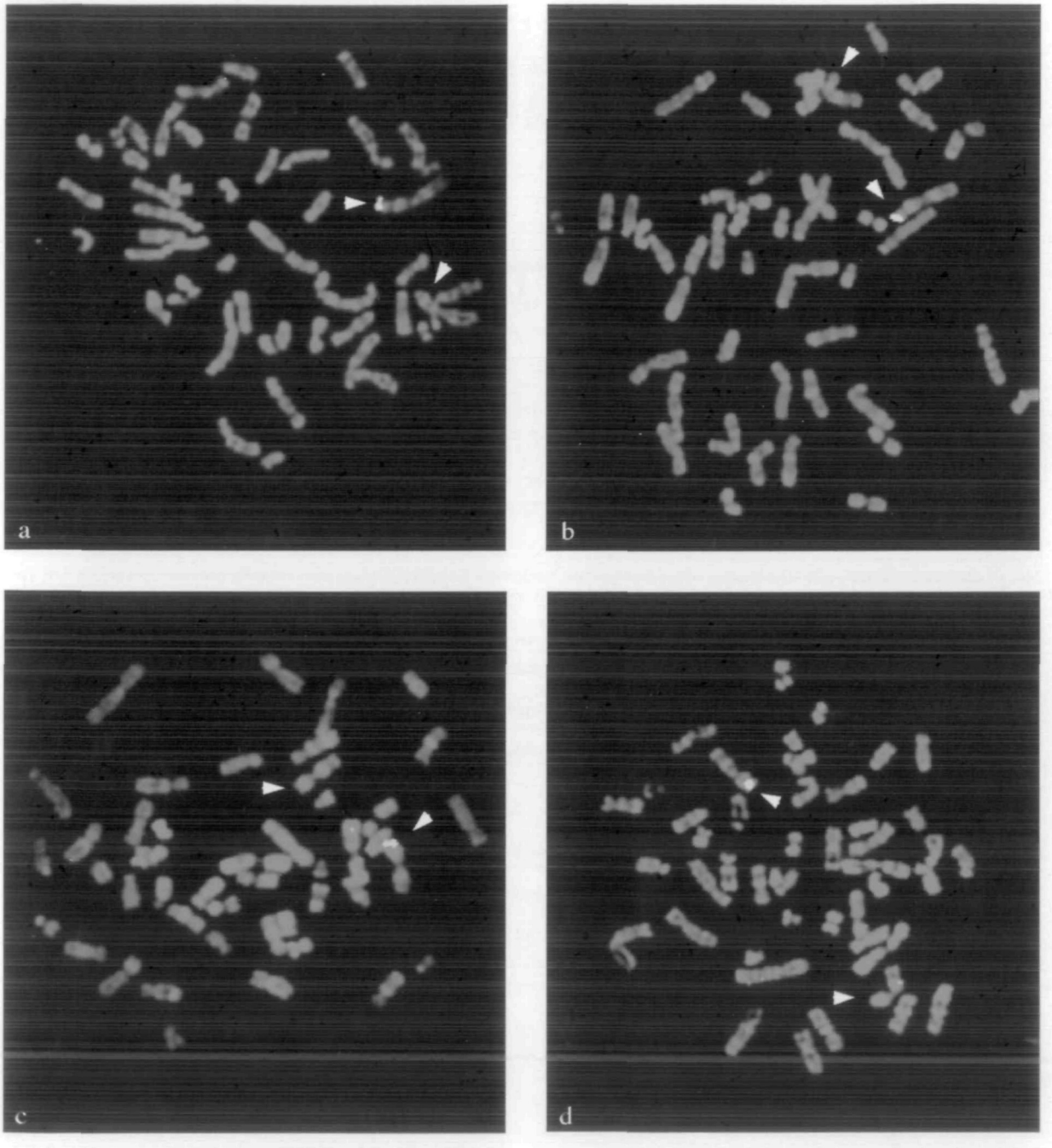

Figure 2. FISH studies in four patients with 3p− syndrome. In each case there is a hybridization signal from the normal chromosome 3pter but not from the deleted homologue. (a) CUMG3.1 and D3S1442, (b) GM10922 and D3S1443, (c) GM10985 and D3S1442, (d) CUMG3.4 and D3S1442.

interstitial deletion of chromosome 3p25−p26 between RAF1 and D3S17 (which maps 19 cM telomeric to D3S18) displayed typical features of 3p− syndrome. Thus the critical region for the expression of classical 3p− syndrome appears to be a 21 cM interval between D3S1317 and D3S17 (see Figure 1). An intriguing finding was the possible relationship between the extent of the 3p deletion and the presence of congenital heart disease in 3p− syndrome. If the observed association between cardiac septal defects and more extensive 3p deletions is causal, then a gene involved in normal cardiac development should be contained between D3S1250 and D3S18. Three genes have been isolated from this region: (i) plasma membrane calcium transporting ATPase isoform 2 gene (PMCA2) (16); (ii) g6 a cDNA of unknown function; and (iii) the VHL disease gene (6). VHL disease patients with large germline deletions including both the VHL gene and g6 do not have congenital heart disease (6), but the PMCA2 gene (which is not deleted in these patients) may be a candidate gene for the occurrence of cardiac septal defects in 3p− syndrome.

Although VHL disease has not been reported in 3p− syndrome patients, the VHL gene was deleted in a least three of five cases we investigated. The absence of VHL disease in these patients

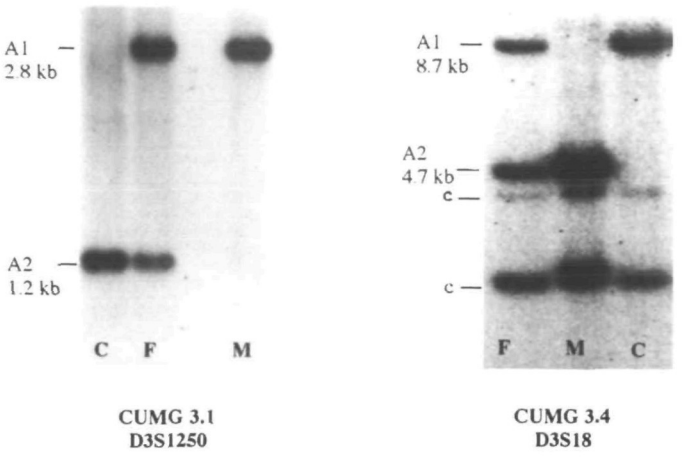

Figure 3. Maternal origin of distal 3p deletion in two patients with 3p− syndrome. For CUMG3.1 the affected child (C) has failed to inherit the A1 allele at D3S1250 from the mother (M), but has inherited A2 allele from the father (F). For CUMG3.4 the affected child (C) has failed to inherit the A2 allele at D3S18 from the mother (M), but has inherited A2 allele from the father (F). (c = constant band).

reflects the young age of the children investigated (the penetrance of VHL disease is 0.05 at 10 years; 20). Early detection of retinal angiomatosis and renal cell carcinoma in VHL disease patients reduces morbidity and mortality (21). Therefore 3p− syndrome patients found to be deleted for the VHL gene should be screened for evidence of VHL complications with an appropriate screening programme (20). Further molecular investigations will identify the minimal deletion necessary for expression of the 3p− syndrome and provide further information on the relationship between the molecular pathology and the clinical phenotype.

PATIENTS AND METHODS

Patients

Five patients with cytogenetic deletions of chromosome 3p25−p26 (del(3)(qter−p25:)) and normal parental chromosomes were investigated. The clinical features of four patients have been reported in detail previously: CUMG3.1 (7), CUMG3.10 (1), GM10985 and GM10922 (cases 1 and 2 respectively in reference 3). For these patients a summary of the clinical features was prepared from the published reports and updated by re-examination of the affected child in two cases (CUMG3.1 and CUMG3.10). Lymphoblastoid cell lines were available from four patients (CUMG3.1, CUMG3.4, GM10985 and GM10922) and DNA from the affected child and both parents in three families (CUMG3.1, CUMG3.4 and CUMG3.10). Lymphoblastoid cell lines for cases GM10985 and GM10922 were obtained from the NIGMS Genetic Mutant Cell Repository, Camden, New Jersey, USA.

FISH analysis

Molecular cytogenetic studies were performed by FISH on metaphase chromosome spreads prepared from EBV transformed cell lines as described previously (8). Cosmid probes were biotinylated by nick translation with biotin-11-dUTP (Sigma). Chromosomal *in situ* suppression (CISS) hybridization was performed to improve the specificity of hybridization with unlabelled sheared human placental DNA added to the biotin-labelled probe at a ratio of 50−100:1. Hybridization signals were visualized by two-layer avidin fluorescein isothiocyanate (FITC) detection system (9) and analysis was carried out under a confocal laser scanning microscope (MRC 600; Bio-Rad Microscience Ltd) with a 100×Plan Apo objective lens (Nikon) and a Nikon Optophot fluorescence microscope. The images were photographed using Kodacolor Gold film.

Metaphase spreads were investigated with cosmid probes at RAF1, D3S651, D3S1250, D3S587, D3S601, D3S18, D3S1442, D3S1443 and D3S1444 (see Table 3). The three cosmid probes for D3S1442, D3S1443 and D3S1444 detect chromosome 3p-specific subtelomeric sequences (10; Dietz-Band unpublished observations). The results of FISH analyses for cases CUMG3.1 and CUMG3.4 at RAF1, D3S651, D3S732, D3S1250, D3S587, D3S601 and D3S18 were included in a previous physical mapping paper (8), and Mowrey *et al.* (5) have previously studied GM10922 and GM10985 at RAF1, D3S18 and D3S17.

Molecular genetic studies

DNA was available from the parents of CUMG3.1, CUMG3.4 and CUMG3.10. These families were analysed using restriction fragment length polymorphisms or microsatellite polymorphisms at RAF1, D35732, D3S601, D3S18 (11), D3S1250 (12), D3S1038 (13), D3S1317 (14) and D3S1444 (Dietz-Band in preparation; see Table 3) to confirm the results of FISH analysis and to establish the parent of origin of the chromosome 3p deletions.

Background map

The order of loci in chromosome 3p25−p26 is shown in Figure 1, and was derived from a variety of studies (6,11,12,15−18).

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